

Drugs versus bugs: in pursuit of the persistent predator *Mycobacterium tuberculosis*

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Abstract | Tuberculosis (TB) claims a life every 10 seconds and global mortality rates are increasing despite the use of chemotherapy. But why have we not progressed towards the eradication of the disease? There is no simple answer, although apathy, politics, poverty and our inability to fight the chronic infection have all contributed. Drug resistance and HIV-1 are also greatly influencing the current TB battle plans, as our understanding of their complicity grows. In this Review, recent efforts to fight TB will be described, specifically focusing on how drug discovery could combat the resistance and persistence that make TB worthy of the moniker 'The Great White Plague'.

Many think of tuberculosis (TB) as the scourge that devastated Europe in the seventeenth century and rapidly became a leading cause of death worldwide before being virtually eliminated (BOX 1). TB has been brought back to our thoughts, however, by the recent reports of outbreaks of drug-resistant disease. In fact, TB never 'went away', but has remained the 'Captain of Death' throughout much of Asia and Africa. TB mortality rates are once again on the rise — this has been attributed to the HIV-1 epidemic, which has produced a new and highly susceptible population, and the inconsistent use of antibiotics, which has led to a new epidemic of drug-resistant disease in many parts of the world. Given that investment in antibiotics in general, including antitubercular drugs, has waned over recent years, we have found ourselves unable to respond to the resurgence of TB.

Two factors, persistence (BOX 2) and resistance, have made the treatment of the causative organism, *Mycobacterium tuberculosis*, difficult. The term persistence describes the survival of *M. tuberculosis* despite the use of antibiotics (rather than latency, which refers to the ability of apparently dormant bacteria in asymptomatic infected individuals to activate as much as decades after the initial infection). Little concrete information is available on the cellular or metabolic status of persistent mycobacteria. As a consequence of persistence, drug treatment is extended, and current antibiotics (BOX 3) require long courses of treatment to cure patients and prevent relapse. Currently, even the most effective regimens require a combination of at least 3 drugs and last

for 6 months. As patients feel better within 1–2 weeks, they have little motivation to complete therapy. Thus, current World Health Organization guidelines call for treatment to be directly observed. The required infrastructure for drug-delivery and treatment supervision can be difficult to provide in much of the world, particularly in areas that are afflicted with poverty and unstable governments.

For TB, drug resistance is due to genetic mutations that result in a heritable loss of susceptibility to antibiotics. These mutations generally occur either in the target or the activator of the drug. TABLES 1,2 summarize the most common mutations for current first- and second-line drugs, and refer to their respective mechanism (or mechanisms) of action and half-lives. Slow-acting drugs, combined with poor health-care systems, have led to incomplete treatment, relapse and the emergence of resistant mycobacteria. Strains of *M. tuberculosis* that are resistant to at least one of the first-line antibiotics (TABLE 1) have become common, and strains that are resistant to two or more are not uncommon. Although resistance to single agents generally still permits cure (albeit by using even more extended courses of therapy), strains that are resistant to multiple agents cause disease that is far more difficult and costly to treat. This is particularly true for multidrug-resistant (MDR) strains that are resistant to the first-line drugs isoniazid (INH) and rifampicin. Some strains carry far greater levels of drug resistance, including extensively drug-resistant (XDR) bacteria, which are MDR and also resistant to fluoroquinolones

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Box 1 | **Waksman's vision**

In his speech at the Nobel Banquet in December 1952, Selman Waksman said "The Great White Plague, which only 10 years ago was thought to be immune to drug therapy, is gradually being eliminated...streptomycin pointed a way. Later supplemented with PAS and more recently with isoniazid, it has brought the control of this disease within sight."

In 1943, Selman Waksman, a microbiologist at Rutgers University, purified a compound from the soil bacterium *Streptomyces griseus* that could kill a wide spectrum of bacteria, including *Mycobacterium tuberculosis*, in culture and in animals^{125,126}. Unlike the discovery of penicillin 15 years earlier, Waksman's discovery of streptomycin was not accidental. His student, Albert Schatz, conducted a directed screen of 10,000 cultures of soil bacteria to identify those that inhibited the growth of co-cultured Gram-negative bacteria. He found only ten cultures that could significantly block the growth of the test bacteria. The Nobel-Prize-winning discovery was heralded by most as the 'beginning of the end for tuberculosis (TB)', although the prize itself has been controversial as some have argued that it should have been shared with his student, or with Jorgen Lehmen, who discovered the TB drug *para*-aminosalicylic acid (PAS) in the same year as the discovery of streptomycin¹²⁷. The drug was quickly approved by the United States Food and Drug Administration, and within 2 years Merck was producing 25,000 kg per day of streptomycin. Although the discovery of streptomycin proved that a bacterium was the cause of the disease and led to almost miraculous responses in patients infected with TB, it required repeated injection and was associated with significant toxicity. Worse, strains of *M. tuberculosis* that were resistant to the drug were discovered within a few months of use.

and at least one injectable antibiotic. These infections are extraordinarily difficult to treat using the current agents.

Clearly, our current drug armamentarium (TABLES 1,2) has not been sufficient to control the TB epidemic (BOX 4). New antibiotics, particularly those that are derived from new chemical classes, are more likely to have activity against many drug-resistant strains. The path to creating antibiotics that act against persistent organisms and produce more rapid clearance of infections is less clear. Certainly, a vital part of drug development is the understanding of the physiology of growing and persistent organisms, an area in which little information is available, as reviewed elsewhere¹⁻³. A greater knowledge of persistence could lead to a directed strategy for the development of more rapidly effective antibiotics. However, until a clear picture of persistence and its role in infection is achieved, researchers will have to rely on more empirical approaches, as demonstrated by the discovery of TMC207 (discussed below).

Here, we review recent and ongoing efforts to produce new antitubercular drugs, and the properties of current investigational agents. This Review seeks to complement other recent discussions of TB research, such as those by Janin⁴, Ginsberg and Spigelman⁵, and Williams and Duncan⁶. We will follow a 'drug timeline', beginning with a discussion of discovery technologies that were designed to provide new clinical antitubercular candidates, before considering compounds that are currently in trials and, finally, already approved drugs that are sought to be used as TB therapies.

Drug-discovery methods

Genetic approaches to target identification. Ideal TB drug targets should have three characteristics: they should be required for bacterial growth and persistence (that is, they must be expressed and essential during the time that treatment occurs); it should be possible to inhibit their activity using small molecules (that is, the target should be 'druggable')^{7,8}; and they should be accessible to these modulatory compounds. Theoretically, the simplest way to find targets that have these ideal characteristics is to discover an active compound and then

define its target. In practice, however, this has not been so simple, and we still do not know the targets of many existing antitubercular drugs that are in clinical use.

Recently, two systematic approaches have emerged to define the targets of compounds that have activity against *M. tuberculosis*. Expression analysis provides a method to profile cellular responses to perturbations, such as small molecules or environmental stress. Several groups have collected a large number of datasets from DNA microarray experiments that were performed under various conditions. In particular, Boshoff and colleagues⁹ have evaluated bacterial gene expression in response to a number of drugs, toxins and environmental conditions. Although these results do not define a single molecular target, they can be used to identify susceptible pathways that may contain drug targets.

The recent availability of affordable whole-genome sequencing also potentially provides a rapid way of finding targets. For example, Andries and colleagues¹⁰ selected for mutants that were resistant to the drug TMC207 (discussed below) and sequenced their entire chromosomes. All resilient strains contained mutations in a single gene that encoded a subunit of ATP synthase. However, this success story is not always easily replicated.

Box 2 | **Bacterial persistence and treatment failure**

Why does antibiotic treatment fail even when bacteria are not genetically drug resistant? This phenomenon, often termed bacterial 'persistence', might be explained in a number of ways, given that *Mycobacterium tuberculosis* induces a chronic inflammatory response in which bacteria are sequestered from drugs in tissue. The local concentration of antibiotics in lesions, such as granulomas, might not be adequate to cause bacterial death, or some bacteria might adopt a physiological state that renders them less susceptible to antibiotics. This could be a stochastic process, in which a subpopulation of cells adopt a physiological state that renders them drug insensitive. Alternatively, an environmental condition, such as low oxygen or carbon starvation, might induce the persistent state. All of these hypotheses could be true. However, as yet, we do not know if any of them have an important role in treatment failure.

Box 3 | Lessons from current antitubercular drugs

There is an excellent chance that patients who have tuberculosis (TB) can be cured using currently available drugs if they are able to complete the required course of therapy. But what characteristics should new drugs have to improve on current treatment?

Oral bioavailability

- Streptomycin is a highly potent drug but is rarely used, partly because it must be injected.

Good tolerance

- *Para*-aminosalicylic acid (PAS) was largely abandoned early on because of gastrointestinal intolerance.

Usability in multiple populations

- Fluoroquinolones are not recommended for the treatment of pregnant women and young children — two populations that are at risk for disease.
- Thiacetazone is associated with life-threatening reactions in patients infected with HIV-1.

Compatibility with antiretrovirals

- Rifampicin alters the metabolism of multiple drugs, particularly protease inhibitors.

Infrequent dosing

- Prospective antibiotics, such as linezolid, might not be useful if they require more than a single dose each day.

Activity against drug-resistant strains

- Drugs such as PAS have been revived owing to the increase in multidrug-resistant and extensively drug-resistant disease.

Activity that is not necessarily *in vitro*

- Pyrazinamide is a highly effective drug for patients even though it has poor activity under standard laboratory conditions.

Rapid clearance of chronic infection

- All available drugs, with the exception, possibly, of rifampicin, have limited efficacy in chronic infection. This is particularly true of agents such as isoniazid that act on the cell wall.

Affordability

- Drugs that are used for other purposes, such as linezolid, are extraordinarily expensive. At current prices, it would be impossible for them to be used in most areas of the world in which TB is prevalent.

In the case of PA-824, sequencing the genome of resistant mutants using a DNA microarray method showed that mutations in a conserved hypothetical gene produced resistance¹¹. However, the encoded protein was not the target of PA-824, but instead was a nitroreductase that was responsible for the activation of PA-824.

Genetic analysis might prove to be an alternative method for defining new TB drug targets. Using a range of methods, investigators have found several gene products that, if inhibited, could decrease bacterial growth or increase host survival after infection^{12–14}. Genes that are required for growth *in vitro* are difficult to define using traditional genetic methods, as mutations in these genes, by definition, result in clones that are unable to grow. However, negative screens that identify genes that cannot be mutated have yielded a set of several hundred candidate genes that are required for *in vitro* growth^{14,15}. Of course, these are simply screens and do not represent proof that individual gene products are useful as drug targets. The extension of these screens to *in vivo* animal models of TB would be a giant step forwards in target identification. However, to validate putative targets in the absence of a known inhibitor it is useful to construct conditional mutants. Fortunately, recent work has provided conditional promoters that can be used to construct these informative strains^{16–18}.

Is it truly important to define single targets for potential antibiotics? After all, it might be difficult to evolve resistance to drugs that hit multiple targets (so-called

dirty drugs) and, in fact, there is evidence that some current drugs, such as INH^{19,20} and *para*-aminosalicylic acid²¹, are capable of inhibiting multiple proteins. However, even in these situations, it is unclear if this provides a strong advantage as resistance can still arise from mutations that seem to affect only single putative targets^{22,23}, perhaps because these represent the points of greatest vulnerability. In any case, for drugs that hit either single or multiple targets, it might be difficult to identify targets using systematic approaches.

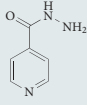
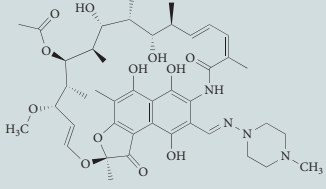
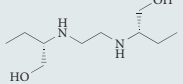
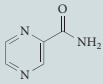
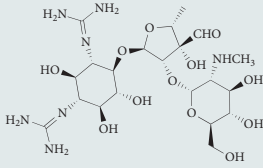
High-throughput screening. High-throughput screening against target proteins has an important role in modern drug discovery^{24,25}. For *M. tuberculosis*, biochemical high-throughput screening has contributed to the finding of two clinical drug candidates, TMC207 (REF. 10) and SQ109 (REF. 26), and has been used to investigate the viability of small molecules as modulators of a number of mycobacterial targets.

Two different approaches — the targeting of whole-cell growth and enzyme inhibition — have been used individually and together to apply high-throughput screening to TB drug-discovery efforts. Whole-cell

Box 4 | Gates' vision

Bill Gates commented at the 2005 World Health Assembly: "Today, we have tuberculosis drugs you have to take for 9 months. Why can't we find one that works in 3 days?"

Table 1 | Mechanism of action, resistance and half-life of current first-line antituberculosis agents

Antibiotic	Chemical structure	Mechanism and target	Mutations associated with resistance	Half-life in humans (hours)	Refs
Isoniazid		Inhibits mycolic acid synthesis; primary target is InhA and secondary targets are KasA and DfrA	<i>katG</i> (required for drug activation); <i>inhA</i> (promoter mutations); and others	1–3	74
Rifampicin		Inhibits transcription; RNA polymerase β -subunit	<i>rpoB</i>	2–3	74
Ethambutol		Inhibits arabinogalactan synthesis; possibly EmbB	<i>embB</i>	3–4	74
Pyrazinamide		Unknown (possibly inhibits FAS-I or alters membrane energetics)	<i>pncA</i> (required for drug activation)	10	74
Streptomycin		Inhibits protein synthesis; 30S ribosomal subunit	<i>rpsL</i> and <i>rrs</i>	2–3	74

screening against either *Mycobacterium smegmatis* or *M. tuberculosis* allows searching for the ultimate goal — potent growth inhibition or killing. As this type of screen is not target based, there is a considerable risk of finding compounds that have generalized toxicity, and the lack of information on the target will certainly complicate the optimization. This risk might be decreased by pre-filtering compound libraries^{27,28} or designing more targeted screens.

High-throughput screening is being pursued at a number of facilities, both in industry and academia. One large National Institutes of Health-funded effort, the *Tuberculosis Antimicrobial Acquisition and Coordinating Facility* (see Further information), offers a free service to investigators for testing candidate compounds. The facility has evaluated over 79,000 compounds from more than 9,600 researchers for the inhibition of *M. tuberculosis* growth²⁹. Of these compounds, 130 have demonstrated *in vitro* efficacy against both drug-sensitive and drug-resistant strains. Such broad screening efforts hold promise for uncovering new leads for antituberculars that, from the outset, display mycobacterial growth inhibition.

High-throughput screening has also been used to identify inhibitors of a targeted enzyme in a cell-free environment, through either a binding or functional assay. High-throughput screening has the potential to identify hits that could be potent inhibitors of mycobacterial growth if the compounds have acceptable

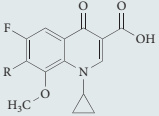


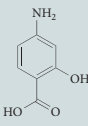
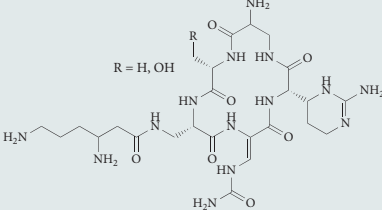
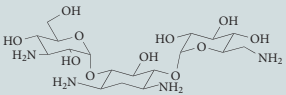
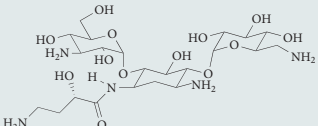
pharmacokinetic profiles and the target is truly essential. There are many examples of small molecules that have excellent enzyme inhibition but poor whole-cell potency, possibly owing to their failure to permeate the mycobacterial cell wall. A variant of this strategy relies on a functional assay in which the inhibition of an entire pathway can be screened for. For example, a luciferase reporter can be used to measure the transcription of the *iniBAC* operon, which is induced by a diverse set of mycobacterial cell-wall biosynthesis inhibitors, including ethambutol and INH³⁰. Barry and colleagues²⁶ used this technology to discover SQ109 (FIG. 1), an ethambutol analogue for which the discovery and clinical progress will be discussed below.

GlaxoSmithKline and the Global Alliance for TB Drug Development (TB Alliance; see Further information) have recently conducted a million-compound screen for new inhibitors of an *M. tuberculosis* enoyl-acyl carrier protein reductase, InhA, which is the target of INH. They found a high hit rate, probably because the crystallographically characterized active site can accommodate hydrophobic groups of varying dimensions, which is consistent with its acceptance of C16–C56 fatty-acid thioester substrates^{31,32}. Importantly, these inhibitors should be active against most of the INH-resistant strains, as they do not require activation by the catalase–peroxidase enzyme KatG³³, which is required for the activation of INH. Similar screens carried out by GlaxoSmithKline and the Novartis Institute for Tropical Diseases for inhibitors of isocitrate lyase, an

Pharmacokinetic profile

A quantitative description of the fate of a drug from the moment the treated subject is dosed with the compound to the moment when it (and/or its derivatives) is expelled from the subject.

Table 2 | Mechanism of action, resistance and half-life of selected second-line antituberculosis agents

Antibiotic	Chemical structure	Mechanism and target	Mutations associated with resistance	Half-life in humans (hours)	Refs or sources
Fluoroquinolones		Inhibits DNA gyrase	<i>gyrB</i>	Moxifloxacin: 12; Gatifloxacin: 8	129
Ethionamide		Inhibits mycolic acid synthesis; <i>InhA</i>	<i>ethA</i> (required for drug activation) and <i>inhA</i> (promoter mutations)	2	130
Cycloserine		Inhibits peptidoglycan synthesis by blocking the synthesis and use of D-alanine (Ala); Ala racemase and D-Ala-D-Ala ligase	<i>alr</i> (overproduction) and <i>ddl</i> (overproduction)	10	DrugBank (see Further information)
Para-aminosalicylic acid		Inhibits folate metabolism; possibly dihydropteroate synthase	<i>thyA</i>	0.75–1	131
Capreomycin		Inhibits protein synthesis; methylated nucleotides in both ribosomal subunits	<i>tlyA</i> and <i>rrs</i>	4–6	The Merck Manuals Medical Library (see Further information)
Kanamycin		Inhibits protein synthesis	<i>rrs</i>	2	132
Amikacin		Inhibits protein synthesis	<i>rrs</i>	3	133

enzyme that is crucial for bacterial persistence during infection^{34,35}, were considerably less successful. Again, this might be explained structurally, as the active site of this enzyme is shallow and highly charged. Small, hydrophilic molecules, such as those that are predicted to bind to isocitrate lyase, are under-represented in most screening libraries, as they are less likely to have desirable pharmacological properties. Finally, a high-throughput screen for inhibitors of pantothenate synthetase, an enzyme that is crucial for the biosynthesis of the essential cofactors acyl carrier protein and coenzyme A, was recently reported by White and colleagues³⁶. Of approximately 4,000 compounds assayed, one lead compound was identified and a preliminary structure characterized in complex with the target enzyme. This successful outcome suggests a path forward for the structure-based design of more potent analogues that inhibit the enzyme and *M. tuberculosis*.

Combining whole-cell and target-based screens might avoid the drawbacks of each. For example, the

library that was used to discover SQ109 was screened using both growth-inhibition and cell-wall-biosynthesis assays³⁷. Of nearly 5,000 compounds screened, 25 small molecules were active in both screens, but only one, SQ775, showed significant activity in a mouse model of infection.

Structural biology and virtual screening. Describing the proteome of *M. tuberculosis* has been the focus of much research in the past few years. Primarily owing to the efforts of the TB structural-genomics consortium, more than 260 X-ray crystal structures of interesting proteins, a large percentage of which were selected on the basis of their being potential drug targets, have been completed, and many are currently being used to facilitate medicinal-chemistry efforts to rationally design new antibiotics³⁸ (FIG. 1). The availability of these structures provides the opportunity to carry out virtual screening. This

Pharmacophore

The chemical functional group (or groups) that is present on a molecule and that enables its biological activity.

Chemotype

A chemical functional group or classification of a specific array of functional groups.

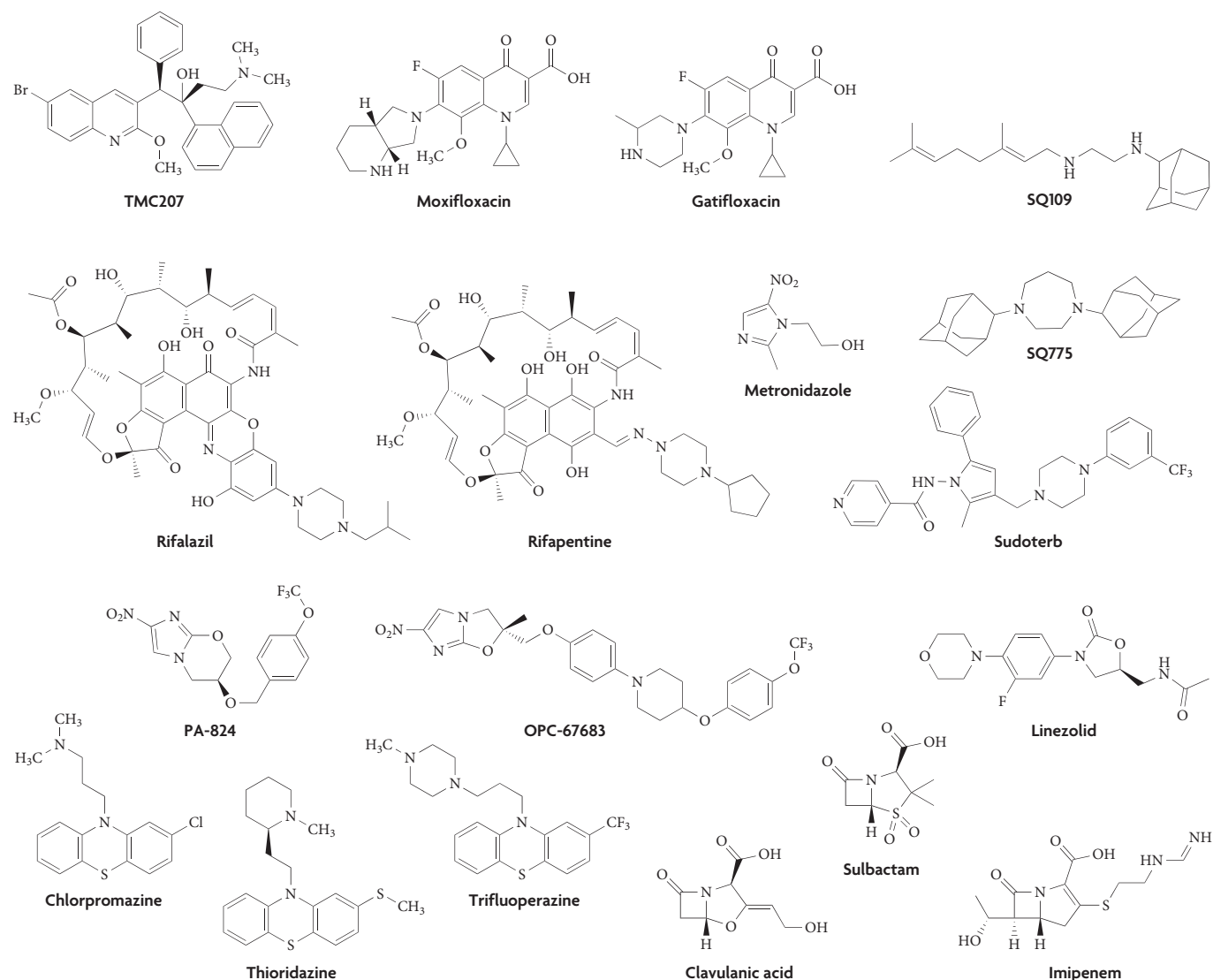


Figure 1 | Chemical structures of non-approved antituberculars.

Lipinski's rules

A set of delimited physiochemical properties described by C. A. Lipinski that best fit a studied subset of drugs. In general, compounds that adhere to these guidelines are said to be drug-like.

Shikimate pathway

A series of biochemical reactions in plants and microorganisms that are involved in the biosynthesis of aromatic amino acids.

powerful technique has had a beneficial impact on numerous drug-discovery efforts^{39–41}. Screening using computational methods can be complementary to a biochemical high-throughput screen because of the potential for screening a larger chemical space quickly and inexpensively. Virtual screening can be used in two ways: to identify compounds that are consistent with a pharmacophore model irrespectively of the identity and structure of the pertinent protein target or targets; or to develop inhibitors of a protein based on its known three-dimensional structure.

Recent examples of virtual screening that were based on known chemotypes include work from Manetti and colleagues⁴² and García-García and colleagues⁴³. Manetti *et al.* used a training set of 471 small molecules that had a range of *in vitro* activities against *M. tuberculosis* to construct a model that could be searched using a virtual library of compounds that was filtered to follow Lipinski's rules⁴⁴. The virtual hits were assayed against *M. tuberculosis*, and the two most potent compounds

had a minimum inhibitory concentration (MIC) of 25 µg per ml. Using a different mathematical model, García-García *et al.* tested 5,000 commercially available compounds and found 18 virtual hits⁴³; 5 of these had an MIC₉₀ of less than 50 µM.

Other groups have instead focused on specific targets that have known structures. Agrawal and co-workers⁴⁵ performed a virtual screen for inhibitors of *M. tuberculosis* chorismate mutase, which is part of the essential shikimate pathway in *M. tuberculosis*⁴⁶. They started with known inhibitors of the homologous *Saccharomyces cerevisiae* enzyme⁴⁷ and conducted a three-dimensional pharmacophore search of a database that has more than 15,000 members. Of the 15 molecules that scored highest, 4 demonstrated inhibition in an enzyme assay. Lin and colleagues⁴⁸ focused on the elucidation of new small-molecule inhibitors of AccD5, an acyl-CoA carboxylase essential enzyme that catalyses the transformation of acetyl-CoA and propionyl-CoA to the corresponding malonyl-thioester and

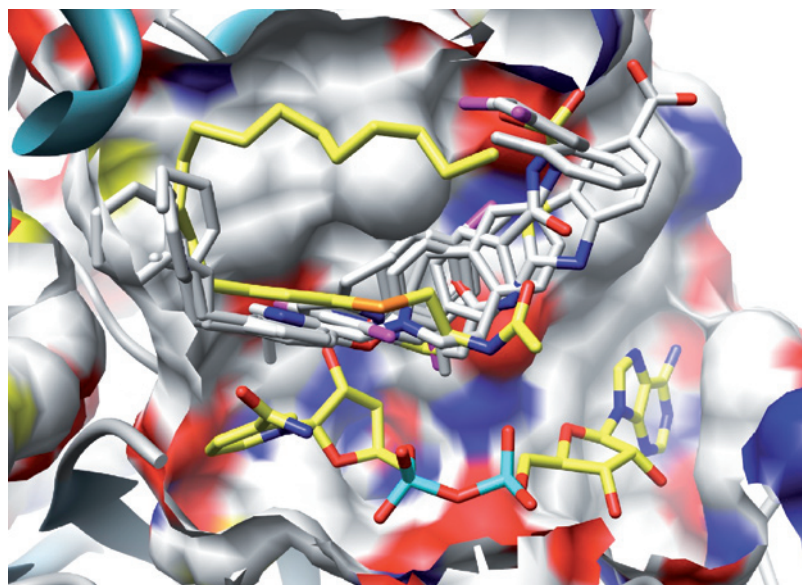


Figure 2 | Overlay of small-molecule inhibitors of InhA. A cross-section through the surface of the active site of *Mycobacterium tuberculosis* fatty-acid enoyl-acyl carrier protein reductase (InhA), coloured by atom type (carbon, grey; nitrogen, blue; oxygen, red; and sulphur, yellow). Superimposed are the bound conformations of NADH⁵⁰ (carbon, yellow; nitrogen, blue; oxygen, red; and phosphorous, aqua), C16 fatty acyl substrate analogue *trans*-2-hexadecenoyl-(N-acetylcysteamine) thioester³², shown in its bent conformation (carbon, yellow; sulphur, orange; nitrogen, blue; and oxygen, red) and 12 inhibitors (carbon, grey; nitrogen, blue; oxygen, red; sulphur, yellow; and fluorine, purple) that have half-maximal inhibitory concentration values ranging from 50 nM to 5 μ M. Unlike isoniazid and ethionamide, these are non-covalent reversible inhibitors that form ternary complexes with enzymes and NAD⁺. This figure was created using the molecular modelling system Chimera¹²⁸.

methylmalonyl-thioester, respectively. They screened over 4 million compounds for binding to either the predicted biotin or propionyl-CoA binding pockets and found that 1 of the 9 compounds that scored highest had an enzyme half-maximal inhibitory concentration (IC₅₀) of 10 μ M. Approaches such as these underscore the usefulness and potential of virtual screening to enable new hits for drug-discovery efforts.

Each of the discovery technologies discussed in this section has potential as a starting point for the discovery of a new antitubercular that, on successful passage through the pre-clinical drug-development pathway, can produce a clinical candidate. Undoubtedly, the most efficient and expeditious way to seed drug discovery lies in the cooperative union of these methodologies in moving from the validated drug target to a clinical candidate. This has been highlighted by examining retrospectively a GlaxoSmithKline antibacterial high-throughput screening campaign, which, over a wide range of targets, provided few compounds for follow up⁴⁹. Additionally, lead optimization is often a labour- and time-intensive process that many programmes do not endure.

A detailed understanding of the drug target (or targets) and how the binding of a drug inhibits target function helps immensely in developing a lead. Whereas efforts to develop new InhA inhibitors, which, in the future, could complement and/or supplant INH, have

yet to yield a clinical candidate, X-ray crystallography and structure-based designs have had crucial roles in the discovery of new inhibitors of this validated TB drug target^{32,50–53}. Sullivan and co-workers⁵⁴ have translated these insights into the discovery of potent triclosan-based antituberculars. FIG. 2 depicts a select subset of X-ray structures that have had a prominent role in this developing story.

New TB drugs in clinical trials

A chemical entity that has been discovered as an antitubercular by the application of one or more of the methods discussed above and has cleared the considerable hurdles in pre-clinical development can enter clinical trials if it has the approval of the pertinent governmental body (in the United States, the Food and Drug Administration (FDA)). The scientific community is hopeful that the drug candidates described below (the structures of which are shown in FIG. 1) will eventually be used in new treatment regimens that meet the goals discussed earlier. It is clearly a testament to the financial commitment of the involved funding organizations that these candidates are being supported through a process that is incredibly costly.

Fluoroquinolones. Fluoroquinolones, which have been used for the treatment of TB since the 1980s⁵⁵, currently constitute the second line of defence against *M. tuberculosis* and play a key part in the treatment of MDR disease. Resistance to fluoroquinolones has been attributed to mutations in the genes that encode gyrase (*gyrA* and *gyrB*)^{56,57}. As quinolone efflux pumps may also have a role in resistance, it is noteworthy that the annotation of putative pumps in the *M. tuberculosis* genome⁵⁸ and the studies of Jacobs and co-workers⁵⁹ on a probable efflux pump, *lfrA*.

Two approved fluoroquinolones, moxifloxacin and gatifloxacin, have shown promising results both for treating resistant disease and, possibly, shortening the course of therapy. Phase II and III clinical trials that are currently underway are testing the efficacy of moxifloxacin and gatifloxacin as replacements for either INH or ethambutol in first-line therapy^{60,61}. These compounds are particularly attractive, as they have already been approved for use in various other infections and are known to be safe. As fluoroquinolones are prescribed for numerous respiratory infections, many cases of TB could be treated with this monotherapy before they are diagnosed. Theoretically, this could lead to drug resistance, although, as yet, widespread fluoroquinolone-resistance mutations have not been observed.

Rifampicin analogues. Rifapentine was approved by the FDA for the treatment of *M. tuberculosis* infection in June 1998. The piperazinyl hydrazone functionalized rifampicin analogue, initially named DL 473, was first reported in 1975 (REF. 62) and subsequently has been shown to exhibit *in vitro* antimycobacterial efficacy that is, in most cases, superior to that of its parent^{63–65}. A noteworthy advantage that rifapentine has compared with rifampicin is its longer serum half-life⁶⁶, which has encouraged its examination in clinical

Lead optimization

The process by which a promising small-molecule entity is structurally modified to obtain drug-like pharmacokinetic, pharmacodynamic and safety profiles.

Efflux pump

An active transport system for the removal of toxic molecules, such as antibiotics, from cells.

settings to determine its potential for positively altering the frequency of the antitubercular treatment regimen. An extension of a Phase III trial that is currently evaluating rifapentine and INH in the treatment of latent TB involves recruiting children (C. Dukes Hamilton, personal communication) to assess the efficacy, pharmacokinetic and safety profiles of the drug combination.

Rifalazil. Rifalazil, formerly known as KRM-1648, has a benzoxazinorifamycin structure⁶⁷. This heterocyclic modification of ansamycin⁶⁸ is much more potent against *M. tuberculosis* clinical isolates than rifampicin⁶⁹ and is more efficacious in a mouse model⁷⁰. Rifalazil presumably has a similar mechanism of action to rifampicin⁶⁸, as mutants are cross-resistant⁶⁹. Rifalazil has a longer half-life than rifampicin in healthy human volunteers⁷¹ and, unlike rifampicin and rifabutin, is neither metabolized by, nor an inducer of, rat and dog hepatic cytochrome P450 enzymes⁷². These metabolic observations suggest that rifalazil can be co-administered with drugs that are sensitive to oxidative metabolism, thereby reducing the potential for adverse drug–drug interactions. This is a particularly important consideration, as co-infection with HIV-1 and TB is common and rifampicin considerably lowers the serum levels of antiretroviral protease inhibitors⁷³. Rifalazil, however, is not currently registered with the FDA for a clinical trial of TB, probably owing to the toxicity that was observed during a Phase II trial in Brazil⁷⁴. ActivBiotics is currently investigating significantly lower once-weekly doses of rifalazil in Phase II/III studies to ascertain the effect on patients that have carotid atherosclerotic disease and have been infected with *Chlamydia pneumoniae* (A. Sternlicht, personal communication). It remains to be seen whether these lower doses, if safe, would be efficacious for TB.

SQ109. SQ109 was discovered using a high-throughput screen of ethambutol analogues that had the dual goals of inhibiting mycobacterial cell-wall synthesis and cell growth in general²⁶. Barry and colleagues²⁶ used the structure–activity relationship (SAR) study results reported by Lederle Laboratories^{75,76} to prepare more than 63,000 compounds. The compounds were assayed for the inhibition of *M. tuberculosis* growth and cell-wall biosynthesis. They found 119 hits in the cell-wall-biosynthesis assay and 60 hits in the growth assay that were at least as potent as ethambutol. SQ109, the optimal hit, shares some structural similarity with ethambutol, but has superior *in vitro* and *in vivo* activity⁷⁷. In fact, DNA microarray analysis suggests that this compound has a different mode of action from ethambutol⁹. SQ109 has achieved fast-track status and is in Phase I clinical trials.

TMC207. Originally named R207910 by Johnson and Johnson Pharmaceutical Research and Development¹⁰, TMC207 is a new antitubercular agent from the diarylquinoline (DARQ) class. The drug was found by a high-throughput screen of a library of approximately 10,000 compounds (K. Andries, personal communication), during a search for those that inhibited the growth of the rapidly growing environmental bacterium

M. smegmatis. It is intriguing to note that the first member of this DARQ class was isolated as a side product in chemical experimentation that was designed to prepare compounds for other early discovery programmes, thus highlighting the importance of screening (targeted compounds and side products) across projects.

TMC207 achieves impressive *in vitro* and *in vivo* efficacy against drug-sensitive and drug-resistant strains of *M. tuberculosis*. Resistance studies identified the biological target as the F_0 subunit of ATP synthase that is encoded by *atpE*^{10,78,79}. This finding, together with the lack of cross-resistance of TMC207 with existing antitubercular drugs, highlights the potential that undirected screens have for the discovery of compounds that have new mechanisms of action. A Phase II trial is currently underway to investigate the efficacy of TMC207-containing regimens versus standard antitubercular therapy in patients who have MDR TB. Given the previously reported animal studies⁸⁰, TMC207 could be added to a second-line treatment regimen or could replace a first-line drug to shorten the length of treatment.

Sudoterb. The tetra-substituted pyrrole Sudoterb was discovered by building on the previously described antimycobacterial SAR of pyrroles⁸¹. The *N*-substituent is similar to INH, as both contain an isonicotinoyl hydrazide moiety. Because the only publicly available information about this compound comes from the patent application⁸², we know little about its mechanism of action. However, the absence of cross-resistance with existing therapies and the unique chemical structure suggests that it could be novel. Sudoterb has been reported to be more potent than INH as a monotherapy in a mouse model for TB and displays an acceptable pharmacological profile in mice and dogs⁸³. The compound is reportedly in Phase I clinical trials⁸³.

Nitroimidazoles. Metronidazole has frequently been used to treat infections of microaerophilic or anaerobic bacteria⁸⁴. Owing to the proposed relevance of limiting oxygen conditions to the latent phase of TB⁸⁵, metronidazole has also been investigated as an antitubercular. Metronidazole kills only dormant *M. tuberculosis* and not actively growing cultures⁸⁶. The drug had no effect on the growth of *M. tuberculosis*-infected mouse macrophages, but a measurable, although small, efficacy in a mouse model of chronic-stage *M. tuberculosis*⁸⁷. Despite these mixed results, a Phase II clinical trial in South Korea is currently recruiting patients to examine the effect of adding metronidazole to a standard second-line therapeutic regimen (see ClinicalTrials.gov in Further information for details of an ongoing clinical trial).

Attempts to discover other nitroimidazole-based antituberculars led researchers at Ciba-Geigy to discover CG-17341 — a nitroimidazooxazole that displays potent activity both *in vitro* and *in vivo* against drug-sensitive and drug-resistant strains^{88,89}. A toxicity issue noted in an Ames mutagenicity test hindered this compound from being developed further. Stover and colleagues⁹⁰ removed this concern, however, with the discovery of the potent antitubercular PA-824 of the nitroimidazopyran

Structure–activity relationship

(SAR). The relationship between the chemical structure of a compound and its biological or pharmacological activity. This type of relationship can be assessed by considering a series of molecules, each with a slightly different structure, and then noting the effect on the biological activity that is associated with each structural variation.

Fast-track status

The FDA status that is reserved for products that demonstrate the potential to treat a serious or life-threatening condition.

F_0 subunit of ATP synthase

The transmembrane portion of the enzyme complex that is involved in the biosynthesis of ATP, which has a role in the passage of protons through the membrane.

Ames mutagenicity test

A sensitive biological method for measuring the mutagenic potency of chemical substances.

class. *In vivo* studies demonstrated comparable activity between PA-824 and INH⁹¹. Most importantly, PA-824 displays no cross-resistance with existing TB drugs and is efficacious against non-growing *M. tuberculosis* under hypoxic conditions. Intriguingly, this activity against persistent mycobacteria is not shared with the original lead compound CG-17341. However, *in vivo* tests using a mouse model have failed to demonstrate an advantage in including PA-824 with first-line antituberculars in terms of shortening the duration of treatment⁹². Mechanistically, the mycobacterial target (or targets) of PA-824 is unknown. Biochemical evidence suggests that both protein and lipid synthesis are inhibited by a compound (or compounds) that is generated from the bio-reduction of the imidazole nitro group^{84,90,93}. Fatty-acid analysis of treated mycobacteria suggested the inhibition of hydroxymycolate oxidation to ketomycolate⁹⁰. Further mechanistic work is required to understand the mechanism of action of PA-824. Overall, the likelihood of a novel mechanism of action and activity against persistent mycobacteria bodes well for the positive impact of PA-824 on antitubercular chemotherapy. This compound is in Phase I clinical trials that are supported by the TB Alliance.

OPC-67683. Building on the work of Ciba-Geigy in nitroimidazoles and the discovery of PA-824, researchers at the Otsuka Pharmaceutical Company in Japan examined the SAR around PA-824 and proposed that further variation of the furan portion of the heterocycle could yield potent antituberculars that are free of mutagenicity^{93,94}. Whereas PA-824 features a pendant 4-trifluoromethoxybenzyloxy group, the Otsuka researchers introduced a 4-piperidine moiety in place of the trifluoromethoxy group to improve oral bioavailability. OPC-67683 was eventually prepared in an effort to decorate the piperidine 4-position with hydrophobic groups such as 4-trifluoromethoxyphenyl.

OPC-67683 is free of the mutagenicity of its nitroimidazole progenitor, is orally bioavailable in mice and provides efficacy that is equivalent to rifampicin against *M. tuberculosis* Kuroko at less than one-fifteenth of the dose⁹³. OPC-67683 was equally efficacious against cultures of drug-sensitive and drug-resistant strains of *M. tuberculosis* and superior to INH, ethambutol, rifampicin, streptomycin, CGI-17341 and PA-824, and also produced rapid eradication of infection in mice. OPC-67683 was not metabolized by a panel of human microsomes and did not positively or negatively interfere with their catalytic activities, thereby lending hope to the idea that this drug could be used in combination with HIV-1 therapies. Mechanistically, OPC-67683 seems to inhibit mycolic-acid biosynthesis and, more specifically, methoxy- and keto-mycolic-acid biosynthesis⁹³, although it probably requires biotransformation for its activity⁹⁵. Given the limited amount of target information that is currently available, it is unclear if OPC-67683 and PA-824 have different mechanisms of action. OPC-67683 successfully completed Phase I clinical trials, demonstrating satisfactory safety and pharmacokinetic profiles in healthy individuals. The small molecule is now being evaluated

for its early bactericidal efficacy in Phase II studies in combination with the standard front-line regimen.

The clinical candidates outlined in this section hold promise as next-generation antituberculars, but each must now pass through the phases of clinical trials to receive approval. This process is not without considerable uncertainty, as demonstrated by the high attrition rates for clinical compounds in the United States.

Approved non-TB drugs as antituberculars

A strategy to reduce the risk that is associated with failure in clinical trials owing to an inadequate human-safety profile lies in the use of already-approved drugs as antituberculars. In this section, we will discuss the potential for three classes of non-TB therapeutics in the fight against TB. The main hurdle has become the demonstration of sufficient efficacy at a dosage level that was previously deemed to be safe.

Linezolid. Linezolid received FDA approval for MDR Gram-positive bacterial infections in 2000. This 3-aryl-2-oxazolidinone antibiotic, and its analogues, has displayed promising *in vitro* and *in vivo* efficacy against drug-sensitive and drug-resistant *M. tuberculosis*^{96,97}. In *Staphylococcus aureus*, and presumably other bacteria such as *M. tuberculosis*, linezolid inhibits protein synthesis by binding to the 23S ribosome to prevent translation⁹⁸. Clinical examination of linezolid as a potential antitubercular, although demonstrating promising efficacy against MDR and XDR TB, has detected significant toxicity^{99–102}. The rate of incidence and severity of these adverse events might be reduced by decreasing the dosage amount of linezolid. A clinical trial that is expected to be completed in late 2007 is currently being conducted in Brazil to determine the efficacy of lower drug doses (J. Johnson, personal communication). In addition, further optimization of the oxazolidinone series for *M. tuberculosis* activity is underway¹⁰³.

The potential for using already approved drugs as antitubercular agents holds considerable promise. Given that marketed drugs have well-documented and acceptable safety profiles, a major hurdle has been removed in terms of finding new therapies for TB. However, as most antibiotics have little activity against *M. tuberculosis*, the crucial step in using these drugs is to demonstrate good efficacy.

β -lactam. Although the β -lactam class of antibiotics has been used in clinics for over 60 years, none of its representatives has been used for the treatment of TB. β -lactams act by inhibiting bacterial-cell-wall biosynthesis and would be a welcome addition to the antitubercular arsenal. Unfortunately, *M. tuberculosis* produces only a single β -lactamase that has broad specificity^{104–106}. Other genes may also have a role in resistance by affecting cell-wall permeability and/or binding affinity for the pertinent penicillin-binding proteins¹⁰⁷. Although β -lactams can penetrate the cell and inhibit their targets¹⁰⁸, their potency is primarily limited by β -lactamase-mediated degradation. Two strategies could avoid this problem. First, in some cases, β -lactamase inhibitors, such as

clavulanic acid and sulbactam, allowed growth inhibition below the $\mu\text{g per ml}$ level if used in combination with β -lactams. Second, the carbapenem imipenem is resistant to cleavage. Unfortunately, both imipenem^{108–110} and amoxicillin in combination with clavulanic acid¹¹¹ have produced mixed results in early bactericidal-efficacy assessments in humans. However, this remains a promising area for investigation and recent structural work using *M. tuberculosis* and *Mycobacterium fortuitum* β -lactamases^{106,112} might prompt the further design of β -lactamase inhibitors and/or β -lactams that have reduced β -lactamase susceptibility.

Phenothiazines. Phenothiazines have a rich and diverse history of medicinal uses¹¹³ and antitubercular activity against drug-sensitive and drug-resistant strains of *M. tuberculosis*^{114,115}. The major examples are chlorpromazine¹¹⁵, thioridazine¹¹⁶ and trifluoperazine^{117,118}. These drugs have substantial, and sometimes even disabling, side effects that limit their use at effective plasma concentrations. However, they might be more effective *in vivo* than *in vitro* as they are concentrated in macrophages^{119,120}.

Phenothiazines appear to target the type-2 nicotinamide adenine dinucleotide (NADH):menaquinone dehydrogenase (NDH-2)¹²¹. NDH-2 is crucial to the mycobacterial electron-transport chain and is the only such enzyme in *M. tuberculosis* that is absent in humans. Notably, this target seems to be important in starved cultures¹²², suggesting that NDH-2 could be

an attractive target for other compounds. One way to achieve this would be to start with the known active phenothiazines and construct analogues that have more selectivity, a goal that is currently being pursued^{123,124}.

Conclusions

M. tuberculosis would seem to be a vulnerable organism, given that it has no noteworthy animal or environmental reservoir and limited genetic diversity. However, despite the availability of several effective antibiotics, TB continues to be a widespread and devastating disease. The need for new fast-acting drugs is clear. Fortunately, several chemical entities are currently in clinical trials, and numerous promising compounds are in the earlier stages of drug development. It is likely that new drugs will become available in the near future that can cope with the resistance problem as it currently exists. However, resistance continually evolves. Persistence is currently an even more formidable enemy, requiring a much more thorough understanding of its basic biological underpinnings and the small molecules that can modulate its role in the disease state. Therefore, little hope exists in the short term for a drastic reduction in the time for treatment, given the targets and drugs that are under clinical investigation. Despite the considerable challenges that are posed by these 'bugs', the recent improvement in the scale of efforts to find new drugs lends hope that science will deliver on the promise of eradicating 'The Great White Plague' that was celebrated prematurely by some owing to Selman Waksman's Nobel Prize.

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 ClinicalTrials.gov: <http://clinicaltrials.gov/ct2/show/NCT00425113?term=Metronidazole&rank=3>
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